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Breast epithelial cell function is greatly influenced by interactions with the underlying basal lamina. Matrilysin, a matrix metallo-proteinase, has previously been shown to be expressed in both adenomas and carcinomas of the human breast. We have tested the hypothesis that cell-ECM interactions regulate the expression of matrilysin in human breast carcinoma cells *in vitro*. The cell line MDA-468 which constitutively expresses matrilysin when plated on plastic was plated various ECM proteins. Northern analysis of cells seeded on RGD peptides, fibronectin, laminin and ECM indicated that matrilysin remained at constitutive levels for >6 hours, but by 20 hours the mRNA declines by at least 50% and reached a nadir of 20% of the initial mRNA levels. Collagen type I inhibited matrilysin production in less than 6 hours and this inhibition was sustained throughout the 48 hour time course. These data indicate that breast tumor cells can regulate matrilysin in response to cues from the ECM as the cells invade the host tissue. We also have used northern analysis and *in situ* hybridization to determine levels and localization of several members of the MMP family in human tumors implanted into nude mouse mammary glands. Tumors from mammary glands injected with the human breast adenocarcinoma cell line MDA-MB-468 were shown to express matrilysin; a MMP that is primarily expressed in normal and neoplastic cells of epithelial origin. Stromelysin-1 was induced in the stroma of the host mammary gland in the region immediately surrounding the tumor. Northern analysis revealed that gelatinase A, which was not produced by MDA-MB-468 *in vitro*, was expressed in the tumor. This combination of MMPs along with stromelysin-3, which has been extensively studied in breast cancer, may lead to the eventual metastasis of mammary tumors to the regional lymph nodes and distant sites.

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William C. Stewart 10/27/95

PI - Signature Date

Table of Contents

Introduction:

Breast cancer is the most commonly diagnosed female cancer in the United States population with an estimated 182,000 new cases in 1993 (32% of all female cancers diagnosed in 1993) (Boring et al. 1993). Metastatic spread to the regional lymph nodes has long been used as a predictor of the extent of disease and with reasonable success. When the tumor has invaded the regional lymph nodes the 5-year survival rate drops from 93% to 73% and when distant metastasis is found the rate falls to 19% (Boring et al. 1993). These data make it clear that the process of tumor cell invasion and metastasis are important in the progression of breast cancer from a benign to a malignant state. The process by which neoplasms metastasize is composed of a complex series of events (Khokha and Denhardt 1989 , Liotta et al. 1991). One of the initial steps in tumor cell invasion is the degradation of the basal lamina (BL) and local invasion of the surrounding tissue. To move through the BL, cells must secrete proteinases that are able to degrade the components of the BL including, collagen type IV, laminin, tenascin and entactin.

The matrix metalloproteinases (MMPs) are a multigene family of secreted proenzymes whose substrates are the proteins that make up the BL and the extracellular matrix (Matrisian 1992 , Powell and Matrisian 1995). Tight regulation of the expression and activity of MMPs and their specific inhibitors, TIMPs, maintain cells in a non-invasive phenotype (Liotta et al. 1991). A change altering the balance in favor of MMP activity may be one of the initial steps in tumor cell invasion. Matrilysin is the smallest known member of the MMP family containing only the signal sequence, the pro-peptide and the active site. Matrilysin has been shown to be expressed in several different human tumor types including: breast, prostate, colon, gastric, rectal and head and neck (For review see (Wilson and Matrisian 1995)). Matrilysin degrades casein, gelatins I, III, IV and V, fibronectin, laminin, elastin and entactin (For review see (Wilson and Matrisian 1995)). Matrilysin also interacts with other proteinases; specifically, matrilysin can cleave activated interstitial collagenase to increase collagenases activity five fold (Quantin et al. 1989). Matrilysin can proteolytically cleave pro-urokinase to yield low-molecular weight pro-urokinase that is unable to bind to its specific transmembrane membrane receptor (Marcotte and Henkin 1993 , Marcotte et al. 1992). Matrilysin mRNA has been shown to be expressed in a high percentage of both adenomas and carcinomas of the breast (Basset et al. 1990 , Wolf et al. 1993). *in situ* analysis indicates that the mRNA is localized to the transformed epithelial cells of the breast (Wolf et al. 1993). I have recently shown that matrilysin expression in prostate tumor cells increases the invasive potential of those cells (Powell et al. 1993).

Mammary epithelial cells have been shown to be highly dependent upon the basal lamina to which the basal aspect of the epithelial cells are attached (Petersen et al. 1992, Talhouk et al. 1992). It has become apparent that changes in the BL or alterations in the way breast epithelial cells respond to normal BL may change the protein expression patterns of the epithelial cells. There is a growing body of literature that indicates that the integrin family of ECM receptors is capable of transducing a signal derived from the ECM to the nucleus and thus alter mRNA and protein production. One of the first instances of signal transduction through an integrin receptor was described by Werb et.al. They showed that proteolytic fragments of fibronectin and Arg-Gly-Asp containing

synthetic peptide fragments can transduce a signal through the fibronectin receptor and increase the expression of Interstitial procollagenase and prostromelysin-1 in rabbit synovial fibroblasts (Werb et al. 1989). It has also been shown that a synthetic peptide derived from the laminin A chain can increase the amount of collagenase IV activity (Kanemoto et al. 1990). Recently it has been shown that both cell adhesion and integrin clustering causes the phosphorylation of a tyrosine kinase ($pp125^{FAK}$) associated with focal adhesion contacts (Juliano and Haskill 1993, Kornberg et al. 1992).

It is of interest to understand the regulation of matrilysin in breast cancer where invasion and metastasis has such a large effect on prognosis. We hypothesize that alterations in the interactions between breast epithelial cells and the BL can modify the invasive and/or metastatic phenotype of the transformed breast epithelial cells. Since the integrin family of receptors are the primary mode of cellular attachment to the BL and they have been shown to transduce signals that effect gene regulation, integrin mediated signal transduction is the most likely method to regulate MMP expression.

Body: Progress on the experiments proposed in this fellowship has been primarily on specific aim 1 which was to:

> Examine the effect of cell/ECM interactions on the expression of matrilysin in normal and neoplastic human mammary epithelial cells that produce and do not produce matrilysin constitutively. Immunoprecipitation and western analysis for matrilysin will be performed on cells plated on plastic, BSA, fibronectin (FN), vitronectin (VN), collagen (Col) and Matrigel.

These experiments have focused on two cell lines, MCF-7 and MDA-MB-468. We have tested both of these cell lines on several matrices including, a fibronectin-like peptide (Sigma), fibronectin, laminin, type I collagen and human ECM. MCF-7 cells do not express matrilysin in standard tissue culture conditions (DMEM, 10% fetal calf serum, 5% CO_2), whereas MDA-MB-468 cells constitutively produce matrilysin under the same conditions. Tissue culture cells were plated on the previously stated matrix proteins, total RNA was isolated, the RNA separated by formaldehyde agarose gel electrophoresis and transferred to a nitrocellulose filter. The human matrilysin and glyceraldehyde-3-phosphate dehydrogenase cDNAs were labeled with ^{32}P and used to probe the northern blot. There was no induced expression of matrilysin in MCF-7 cells in response to any of the matrix proteins (data not shown). Northern analysis of MDA-MB-468 cells plated for 6 to 44 hours on the indicated matrix components is shown in figure 1. The pattern of expression over the 44 hour time course was similar for the fibronectin like peptide, fibronectin, laminin and human ECM. Matrilysin was high during the initial 24 hour period then gradually decreased to the basal level seen after prolonged culture. In cells plated on type I collagen there was no detectable expression of matrilysin over the length of the time course. This effect did not appear to be related to adherence per se, because cells plated on type I collagen attached and spread during the experiment. This indicated that collagen either inhibited the constitutive expression or that the presence of collagen altered the ability of the cells to interact with components of the serum.

The time course in figure 1 lacked very early time points, to determine if matrilysin was expressed earlier than 6 hours after plating on type I collagen the experiment was repeated twice with time points from 30 min. to 72 hours (Figure 2). As can be seen in figure 2, MDA-MB-468 cells on plastic begin to produce matrilysin mRNA as early as 30 minutes after plating. When plated on collagen, MDA-MB-468 cells produced no detectable matrilysin mRNA as late as 72 hours after plating. These cells are attached and viable as determined by trypan blue exclusion (data not shown). The media for this experiment contained serum that had been stripped of fibronectin (by gelatin-sepharose chromatography) to eliminate any effects that could be due to integrin binding of fibronectin. This does not affect the vitronectin content of serum thus these experiments must be considered with the presence of vitronectin in mind.

To determine if matrilysin expression was due to ARG-GLY-ASP (RGD) dependent integrin binding, detached cells were pre-incubated with media containing 1mM RGDS and fibronectin free serum then seeded on plastic, type IV collagen and type I collagen (Figure 3). Cells were adherent within 1 hour on all 3 substrates, with and without RGDS. The northern blots were quantitated using a phosphoimager and corrected for RNA loading with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe. The RGDS peptide was able to inhibit matrilysin expression in cells seeded on plastic and type IV collagen, but had no effect on the cells seeded on type I collagen. This data indicates that binding to RGD is at least partially responsible for matrilysin expression in these cells, but may not be required for cell adherence. A more extensive dose/response time course is necessary to determine the nature of the interaction between MDA-MD-468 cells and RGD containing ECM proteins.

Many tumor cell lines alter their expression of MMPs when implanted in vivo. To determine if matrilysin was expressed in MDA-MB-468 cells in vivo, three previously produced MDA-MB-468 tumors derived from intra-mammary gland injections were screened for MMP expression by in situ hybridization (Figure 4). Panel A shows low power views of hematoxylin/eosin staining, matrilysin *in situ* and a stromelysin-1 *in situ* on serial sections. The H&E showed large areas of necrosis in the center of the tumor; a portion of the mammary gland can be seen in the lower right. The matrilysin *in situ* showed hybridization only in the viable tumor cells. The stromelysin-1 *in situ* showed an intense stromal production of stromelysin-1 mRNA. The intensity was greatest in the cells nearest the tumor with hybridization declining with increasing distance from the host tumor border. Upon closer examination the border between the host and tumor tissue was less defined. Tumor cells (as determined by human matrilysin hybridization) had invaded a short distance into the mammary tissue (Figure 4B), however there was no evidence of distant metastasis. Stromelysin-1 expression was concentrated in the stromal fibroblasts near the tumor as well as in the fibroblasts surrounding a mammary duct near the tumor (Figure 4C). A new MMP recently cloned from a placental library contains a transmembrane domain and was named MT-MMP (membrane-type MMP). MT-MMP is responsible for activation of gelatinase A on the cell surface (Sato et al. 1994). MT-MMP has been shown to be expressed on the cell surface of fibroblasts surrounding mammary carcinoma cells (Sato et al. 1994), but recently MT-MMP was found to be expressed by MDA-MB-231 breast cancer cells in vitro (Yu et al. 1995). *in situ* hybridization of MT-MMP to the MDA-MB-468 tumors indicated that this tumor

cell line expresses MT-MMP mRNA in vivo, although at very low levels (Figure 4D). Northern analysis of RNA from the same three tumors used for in situ hybridization showed expression of both gelatinase A and MT-MMP (Figure 5). The localization of gelatinase A expression has yet to be determined, thus it could be derived from either the tumor cells or the host stroma. This xenograft system has helped to define the complex interactions between host and tumor cell production of MMPs.

Conclusions:

We have shown that matrilysin mRNA is expressed in MDA-MB-468 cells seeded on tissue culture plastic, fibronectin, laminin, type IV collagen and ECM. Matrilysin mRNA is not produced by cells seeded on type I collagen. The expression of matrilysin in cells grown on plastic and type IV collagen can be inhibited by RGDS peptides whereas there is no effect on cells grown on type I collagen. Both stromelysin-1 and collagenase have previously been show to be regulated through integrin receptors interacting with fibronectin fragments and immobilized RGD peptides in fibroblasts (Werb et al. 1989). Matrilysin has recently been shown to be induced by truncated fibronectin in colon adenomas (Yamamoto et al. 1994). In the experiments performed here vitronectin was always present in the FCS and may be partly responsible for matrilysin expression in cells seeded on plastic. Vitronectin contains the RGD cell binding domain. MDA-MB-468 cells seeded on plastic in the absence of FCS do not attach and die within 12 hours, however, in the presence of fibronectin stripped FCS the cells attach and survive. Matrilysin expression in MDA-MB-468 cells plated in the presence of vitronectin alone or with fibronectin was completely inhibited by type I collagen. This could be mediated through any one of the 3 type I collagen integrin receptors, $\alpha 1\beta 1$, $\alpha 2\beta 1$ or $\alpha 3\beta 1$. A recent study using osteosarcoma cells showed that type I collagen inhibited collagen I mRNA production , potentially via the $\alpha 1\beta 1$ integrin (Riikonen et al. 1995). The same study showed an increase in collagenase mRNA in response to collagen I when the $\alpha 2\beta 1$ integrin was present. Future efforts on this fellowship will focus on this interaction between MDA-MB-468 cells and type I collagen and whether $\alpha 1\beta 1$, $\alpha 2\beta 1$ or $\alpha 3\beta 1$ integrins play a functional role in mediating the response as stated in Specific Aim 2.

The xenograft approach has allowed us to answer several questions regarding the effects of host tumor interactions on the production of MMP in the interface between tumor and host tissues. MDA-MB-468 cells *in vivo* express the mRNAs for matrilysin and MT-MMP and potentially gelatinase A. The conflicting information on the localization of MT-MMP could be due to the low levels of expression in the tumor cells. Had this been a mouse tumor in a mouse mammary gland probed with the mouse MT-MMP probe, higher expression in the stroma would have made the tumor expression appear to be only background non specific hybridization. This combination of MMPs expressed by tumor cells could represent a very aggressive phenotype, in fact, MDA-MB-468 cells are metastatic in nude mice. The expression of stromal MMPs in this model was somewhat surprising. Stromelysin-1 being induced strongly near the tumor is a classic pattern for stromelysin-3 in human tumors, but this has not been seen

before for stromelysin-1. This expression could be induced through tumor production of soluble growth factors and cytokines that affect fibroblast MMP production. Alternatively, matrilysin degradation of BL and ECM proteins could release matrix bound growth factors that could be responsible for the stromal production of stromelysin-1. Thus, we have shown that there are several MMPs produced in this model system with novel localizations that function to degrade ECM and BL proteins to allow for invasion with subsequent metastasis. Understanding of these interactions may aid in the evaluation of human breast malignancies for invasive and metastatic phenotypes. This area of research was not proposed in the original fellowship but it complements the information obtained from the in vitro tissue culture studies on cell/ECM interactions. Type I collagen has been shown to induce a gelatinase A activating activity in human breast cancer cells (Azzam et al. 1993, Azzam and Thompson 1992), this activity is presumably the recently described MT-MMP. This could indicate that as mammary carcinoma cells escape the glandular epithelium through the BL and invade the ECM, a change occurs in the expression from matrilysin to MT-MMP which activates gelatinase A produced by the tumor or the stromal fibroblasts of the mammary gland. This work will continue to examine the expression of both human and mouse MMPs by *in situ* hybridization, leading to a more complete understanding of the MMP production at the host/tumor border.

During the first year of my fellowship I attended the 86th annual meeting of the American Association for Cancer Research in Toronto, Canada. While there I presented a poster entitled "The effects of extracellular matrix proteins on matrilysin production in a breast carcinoma cell line, MDA-468" (see appendix 1). I was also accepted to attend the Histopathobiology of Neoplasia workshop organized by AACR. This workshop is designed to train basic scientists in epidemiological, medical and pathological aspects of human cancer. At this workshop attendees are encouraged to present posters of their work, I presented a poster entitled "Matrix metalloproteinase production by human breast cancer cells and host mammary gland stroma in the nude mouse" (see Appendix 2). Dr. Lynn Matrisian and I were asked to write a book chapter this year, and it was entitled "Complex roles of matrix metalloproteinases in tumor progression" (see Appendix 3). This manuscript will appear in a book series titled "Attempts to understand metastasis: metastasis related molecules" published by Springer-Verlag.

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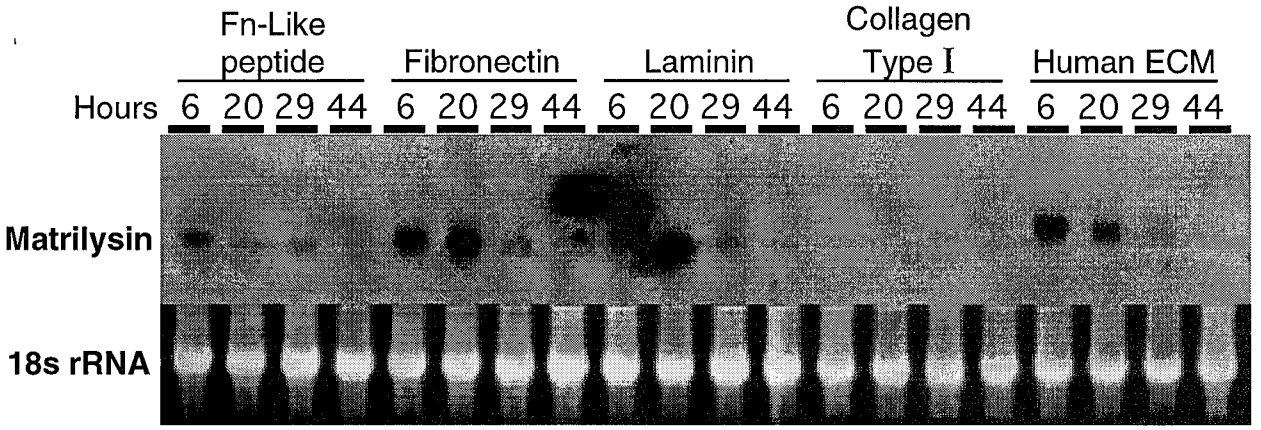

Figure 1. Effects of ECM proteins on matrilysin expression in MDA-MB-468 cells in culture. MDA-MB-468 cells were detached by treatment with PBS-EDTA and plated for the indicated number of hours on each substrate. The plates were coated with 20μg of each substrate prior to the plating of cells. The cells were lysed and the RNA isolated by the acid-guanididium method. Nothern blot analysis was performed using the human matrilysin cDNA. The ethidium bromide staining of the 18s RNA was included to indicate the uniformity of RNA loaded.

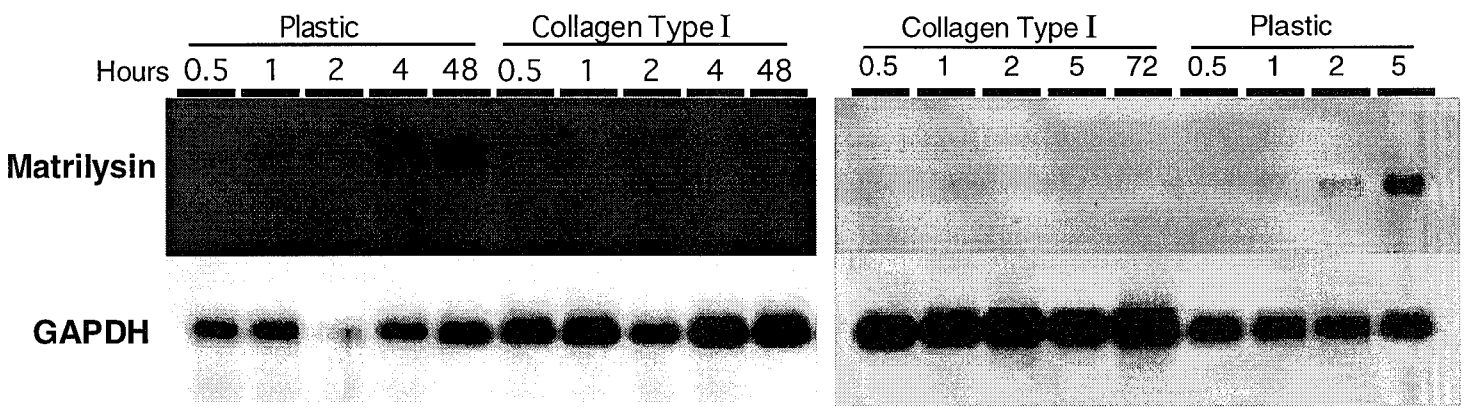

Figure 2. Early effects of plating on plastic or collagen type I on matrilysin expression. The plates and cells were treated as described in Figure 1. The cells were either plated on plastic or collagen type I for the indicated times. The northern blots were probed at high stringency with the matrilysin and GAPD cDNAs.

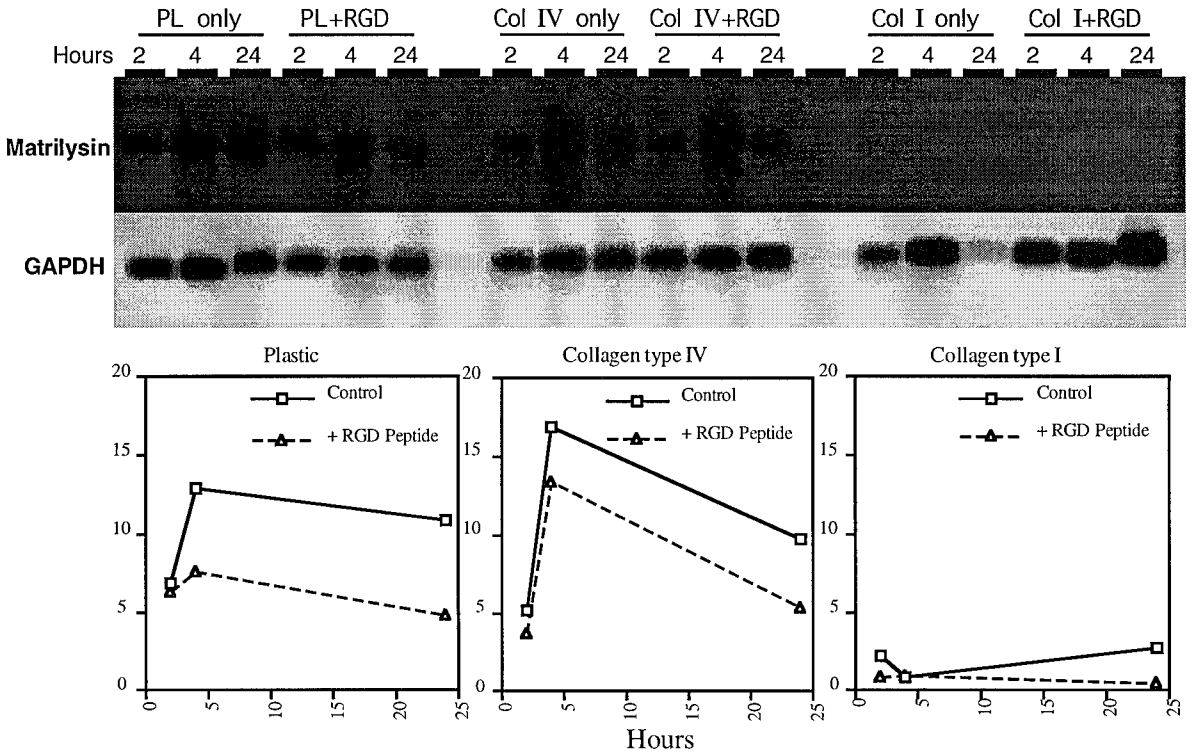

Figure 3. Effect of RGD peptides on matrilysin expression. The plates and cells were treated as described in Figure 1. Cells pre treated with 1μM RGDS peptides were either plated on plastic Collagen type IV or collagen type I for the indicated times in the presence or absence of 1 μM RGDS peptide. The northern blots were probed at high stringency with the matrilysin and GAPD cDNAs. Matrilysin mRNA was quantitated using a phosphoimager and correcting for GAPD levels.

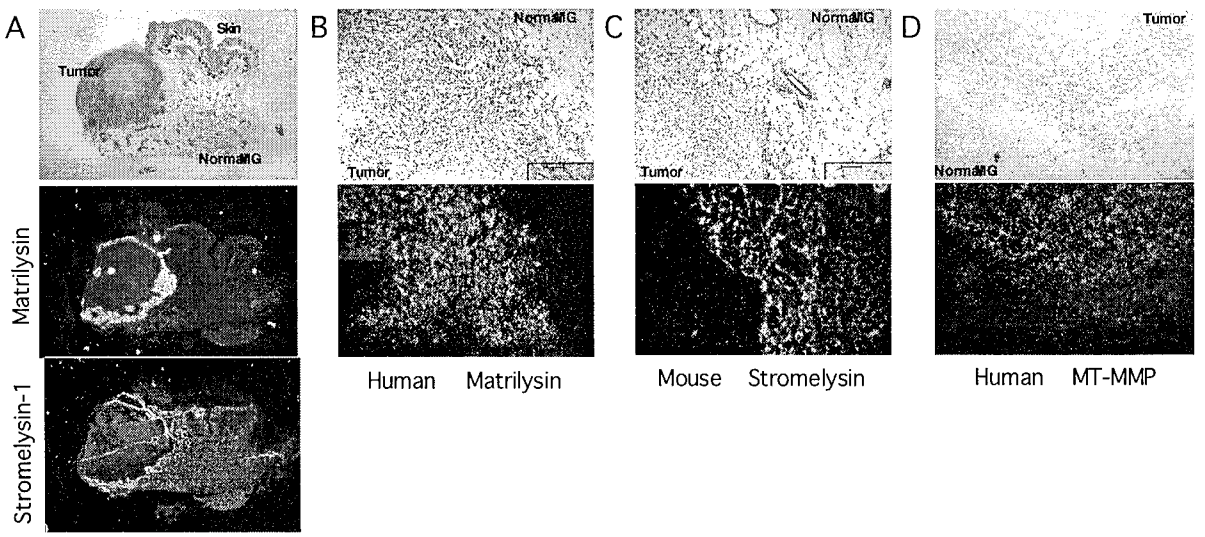

Figure 4. in situ hybridization of MMP probes to MDA-MB-468 tumors in nude mice.
In Situ Hybridization: The in situ protocol used was a modification of a previously described method. Prior to probe hybridization the slides were deparaffinized, post fixed in 4% paraformaldehyde at 4°C for 10 min. and treated with proteinase K for 10 min. The slides were prehybridized at 55°c . Sense and anti-sense matrilysin, stromelysin-1 or MT-MMP riboprobes were labeled with ^{35}S-UTP and used in the in situ hybridization. $1.2x10^6$ cpm of each probe were added to the prehybridization solution and incubated for 18 hours. The slides were washed in high stringency buffer followed by RNAse treatment for 45 minutes. Washes were performed at 65°C in 0.1x SSC, 10 mM β-mercaptoethanol, 1 mM EDTA. The slides were dehydrated and dipped in photographic emulsion (Kodak, NTB type 2) and exposed for 2 weeks. The slides were developed and counter stained with hematoxylin.

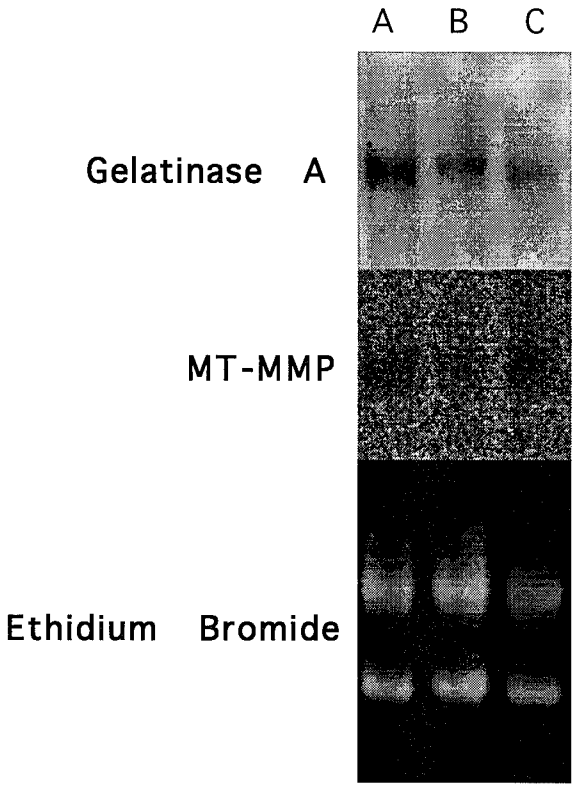

Figure 5. Gelatinase A and MT-MMP mRNA expression in MDA-MB-468 tumors.
Northern anaylsis was performed on RNA from MDA-MB-468 tumors in nude mice. A, B and C are three independent tumors derived from the same cell line. cDNA probes from human gelatinase A and MT-MMP were used as probes and the blot was washed out at high stringency.

APPENDIX 1

The effects of extracellular matrix proteins on matrilysin production in a breast carcinoma cell line MDA-468

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Breast epithelial cell function is greatly influenced by interactions with the underlying basal lamina. Matrilysin, a matrix metallo-proteinase, has previously been shown to be expressed in both adenomas and carcinomas of the human breast. We have tested the hypothesis that cell-ECM interactions regulate the expression of matrilysin in human breast carcinoma cells *in vitro*. The cell line MDA-468 which constitutively expresses matrilysin when plated on plastic was plated various ECM proteins. Northern analysis of cells seeded on RGD peptides, fibronectin, laminin and ECM indicated that matrilysin remained at constitutive levels for >6 hours, but by 20 hours the mRNA declines by at least 50% and reached a nadir of 20% of the initial mRNA levels. Collagen type I inhibited matrilysin production in less than 6 hours and this inhibition was sustained throughout the 48 hour time course. These data indicate that as breast tumor cells can regulate matrilysin in response to cues from the ECM as the cells invade the host tissue. This research was supported by US Army breast cancer grants DAMD17-94-J-4442 (WCP) and DAMD17-94-J-4226 (LMM).

APPENDIX 2

Matrix metalloproteinase production by human breast cancer cells and host mammary stoma in the nude mouse.

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Breast cancer is the most commonly diagnosed female cancer in the United States population with an estimated 182,000 new cases and an estimated 46,000 deaths due to breast cancer in 1993. Metastatic spread to the regional lymph nodes has long been used as a predictor of the extent of disease and with reasonable success. When the tumor has invaded the regional lymph nodes the 5-year survival rate drops from 93% to 73% and when distant metastasis is found the rate falls to 19%. Therefore genes involved in breast cancer invasion and metastasis are of particular interest. The matrix metalloproteinase (MMP) family of enzymes degrade the components of the extracellular matrix and basal lamina, both important barriers to tumor cell invasion. We have used northern analysis and *in situ* hybridization to determine levels and localization of several members of the MMP family in human tumors implanted into nude mouse mammary glands. Tumors from mammary glands injected with the human breast adenocarcinoma cell line MDA-MB-468 were shown to express matrilysin; a MMP that is primarily expressed in normal and neoplastic cells of epithelial origin. Stromelysin-1 was induced in the stroma of the host mammary gland in the region immediately surrounding the tumor. Mouse stromelysin-2 was expressed focally in cells between the necrotic core of the tumor and the viable outer layer of the tumor. Northern analysis revealed that gelatinase A, which was not produced by MDA-MB-468 *in vitro*, was expressed in the tumor. This combination of MMPs along with stromelysin-3, which has been extensively studied in breast cancer, may lead to the eventual metastasis of mammary tumors to the regional lymph nodes and distant sites. This research was supported by US Army breast cancer grants DAMD17-94-J-4442 (WCP) and DAMD17-94-J-4226 (LMM).

APPENDIX 3

Complex Roles of Matrix Metalloproteinases in Tumor Progression

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The local invasion and metastatic spread of tumor cells throughout the body is one of the primary concerns of the oncologist. If all tumors were confined within a defined space, most neoplasms could be removed and subsequently cured with the surgeon's scalpel. However, this is not the case within the scope of cancer biology. Some neoplasms are highly metastatic (melanoma), while others are rarely metastatic (brain tumors), but given enough time and/or negligence most tumors will spread to distant sites. The intent of this series is to understand the mechanisms that tumor cells use to invade, disseminate and form viable metastatic colonies as well as discuss potential therapies for metastatic disease. This chapter will address the role of the matrix metalloproteinases (MMP) and their inhibitors in the process of tumor progression, invasion, and metastasis.

INTRODUCTION

The MMP Multigene Family

The metalloproteinase multigene family is a continually growing group of enzymes that have links to both normal cellular processes, for example the menstrual cycle (Rodgers et al. 1994), and neoplastic invasion and metastasis. The proteinases that comprise the MMP family have several distinguishing characteristics:

(1) the proteins have a characteristic pattern of conserved domains.
(2) the substrates for these enzymes are the proteins that make up the extracellular matrix (ECM) and basal lamina (BL).
(3) proteolytic activity is inhibited by tissue inhibitors of metalloproteinases (TIMPs).
(4) the enzymes are either secreted or transmembrane pro-enzymes that require activation to exert their matrix degrading activity.
(5) the active site contains a zinc ion and requires a second metal cofactor such as calcium.
(6) enzymatic activity is optimal in the physiological pH range.

In the past, the MMP family has been frequently divided into three classes based on their substrate specificity; the collagenases degrade fibrillar collagens, the gelatinases degrade denatured and basement membrane collagens, and the stromelysins degrade proteoglycans and glycoproteins. This classification scheme becomes problematic when new MMPs are cloned or new substrates are identified for an enzyme; this has resulted in the need to place some enzymes in an "other" category until sufficient information is available. We propose a methodology for classifying MMPs based primarily on the protein domain structure. This approach has been used to subdivide other protein families, for example the growth factor receptor/kinase family of proteins. This classification reduces the variability associated with substrate specificity being the determining factor for classification, since not all enzymes have been tested on all ECM and BL proteins, and allows for a more rapid and unbiased classification of new MMP family members.

MMP Protein Structure

The MMP family members share several conserved and easily recognizable protein domains. Under the proposed classification system, the enzymes are categorized based on the number and characteristics of specific protein domains (Table 1). The minimal domain enzyme, matrilysin, contains three domains that are present in all MMPs and make up the structural basis for the entire family. The signal sequence or "pre" domain contains a series of hydrophobic amino acids that direct the protein for export but are rapidly removed prior to secretion. The pro-peptide domain contains a highly conserved segment of eight amino acids, PRCGVPDV, with an unpaired cysteine that interacts with the zinc ion in the active site. This model, termed the "cysteine switch" mechanism of MMP activation (Park et al. 1991 , Springman et al. 1990 , Van Wart and Birkedal-Hansen 1990), was based on the ability of various compounds that activate MMPs to disrupt the interaction between the zinc and the conserved cysteine either directly or by altering the protein conformation of the pro

domain. This event allows a water molecule to be the fourth zinc ligand, thus displacing the cysteine (Birkedal-Hansen et al. 1993). The release of the pro domain from the active site zinc leads to the removal of the pro domain via an auto-catalytic mechanism to produce the mature enzymatic form. The catalytic domain contains three conserved histidines in the peptide sequence HEXGHXXGXXHS. These histidines are required for ligation of the zinc ion and the activity of the MMP (Windsor et al. 1994). The catalytic domain of human collagenase has been crystallized and the structure solved to a 2.0 Å resolution (Bode et al. 1994, Lovejoy et al. 1994). This work verified that the zinc contained in the catalytic site is coordinated by the three histidines of the conserved catalytic domain and that the protein contained a second "structural" zinc. The authors indicated that the structure of the catalytic domain should hold true for the other members of the MMP family with the exception of gelatinases A and B which contain a 182 amino acid insertion (the fibronectin domain, see below) in the catalytic domain.

Matrilysin is the only family member to date that does not contain a carboxy terminal domain that has homology to a heme binding protein hemopexin and the ECM component vitronectin. In this classification scheme, the addition of this domain distinguishes the "hemopexin domain" MMPs from matrilysin (Table 1). Several functions have been assigned to this domain in different MMP family members. The hemopexin domain in progelatinase A and B has been associated with interactions with the inhibitors, TIMP-2 and TIMP-1, respectively (Baragi et al. 1994, Howard and Banda 1991 , Howard et al. 1991 -a, Murphy et al. 1992). In interstitial and neutrophil collagenase, the hemopexin domain is associated with substrate binding (Bigg et al. 1994, Sanchez-Lopez et al. 1993 , Schnierer et al. 1993). The hemopexin domain of gelatinase A is required for cell surface activation (Murphy et al. 1992, Ward et al. 1994).

The hemopexin domain and the catalytic domain are linked by a "hinge region" that is of variable length and composition (Birkedal-Hansen et al. 1993). Interestingly,

this hinge region has a conserved size and peptide sequence in MMPs that are able to degrade fibrillar collagen, and this sequence has been shown to be important in conferring the ability of collagenases to degrade fibrillar collagen (Hirose et al. 1993). This structural difference in the hinge region allows for a subclassification within the hemopexin domain enzymes that differentiates the collagenases from the stromelysins and other MMPs that have similar structures but different substrates.

The most recently described member of the MMP family contains a domain that is common to many proteins but is newly characterized in the MMPs. Membrane-type MMP (MT-MMP) has the domain structure found in the hemopexin MMPs with the addition of a membrane spanning domain. The transmembrane domain of MT-MMP has been verified by deletion and translocation analysis (Cao et al. 1995). Membrane localization is required for its only known activity, activating gelatinase A on cell membrane surfaces (Cao et al. 1995). The recent cloning of MT-MMP has indicated a second potential new domain, one that it shares with stromelysin-3. This domain is a 10 amino acid insertion following the PRCGVPDV sequence that contains the consensus sequence RXKR that is a recognition site for furin-like enzymes (Roebroek et al. 1994). The furin-like enzymes function in protein processing and have been shown to function in a number of protein maturation pathways (Bresnahan et al. 1990). Recently, stromelysin-3 has been shown to be activated intracellularly by furin in COS cells and furthermore, the transfer of the 10 amino acid sequence to interstitial collagenase causes intracellular activation (Pei and Weiss 1995). MT-MMP and stromelysin-3 share another characteristic in that neither enzyme appears to efficiently degrade ECM proteins. Stromelysin-3 requires a C-terminal cleavage as a second activation step and still has very low activity against ECM substrates (Murphy et al. 1993), and the only described substrate for MT-MMP is gelatinase A (Sato et al. 1994). These observations suggest the possibility that the mechanism of activation may affect substrate recognition, or that there maybe other functions associated with MMPs other than degrading ECM and BL proteins directly.

The last subclass of MMPs is based on the presence of the fibronectin domain. Both gelatinase A and B contain a domain that is homologous to the collagen binding region of fibronectin (Collier et al. 1988 , Wilhelm et al. 1989). This region has been shown to be required for gelatinase activity as well as collagen binding of gelatinase A (Murphy et al. 1994). A separate study indicates that the fibronectin domain, when expressed by itself, had a high affinity for gelatin and can displace native fibronectin from gelatin (Banyai et al. 1994). Gelatinase B is the only MMP to contain a sequence similar to the α_2 chain of type V collagen in the hinge region but the functional significance of this insertion is unclear.

We have proposed a new classification system for the MMP family based on protein domain structure. This system allows for faster classification of newly cloned genes and eliminates many of the discrepancies that arose from the substrate specificity-based system. Like their ECM substrates, it is now recognized that the MMPs are a large family of highly modular proteins and that the combination of specific domains relates to the functional characteristics of the individual enzyme.

MMP Activation

It is generally accepted that degradation of the BL or ECM is the end result of a proteolytic cascade involving members of both the serine and metalloproteinase families (Liotta et al. 1991 , Matrisian 1992 , Nagase et al. 1990 , Suzuki et al. 1990 , Woessner 1991). These models are based on the data showing that, in vitro, both MMPs and serine proteinases can act on the pro forms of one another, and on inhibitor studies that demonstrate that both MMPs and serine proteinases are necessary for invasion of tumor and endothelial cells through BL components (Mignatti et al. 1986). Plasmin has been shown to activate most of the MMPs by making one cleavage in the pro-domain which initiates the autoactivation of the MMP (Eeckhout and Vaes 1977, Santibañez and Martínez 1993). The MMP matrilysin can act on urokinase to liberate the amino terminal fragment containing the receptor binding domain from high

molecular weight prourokinase (Marcotte et al. 1992), which could affect subsequent cell-surface activation of urokinase. These activations have been shown to occur in tissue culture (He et al. 1989, Marcotte et al. 1992), but there is little direct evidence that these types of activations occur in vivo. One of the recently discovered MMPs, MT-MMP, has been shown to be responsible for the membrane localized activation of gelatinase A in tumor cells (Sato et al. 1994). The concept of a proteolytic cascade formed cooperatively by different cell types is a subject of considerable interest that requires further investigation.

TIMPs

The activated form of these MMPs can be inhibited by a family of secreted proteins known as tissue inhibitors of metalloproteinases. Currently there are three known TIMPs; TIMP-1, TIMP-2 and TIMP-3. The TIMPs are highly structured proteins that contain 12 conserved cysteines that form 6 disulfide bridges between protein segments (Williamson et al. 1990). The TIMPs are expressed in most tissues and can be co-regulated or differentially regulated with the MMPs depending on the tissue or cell type. TIMP-1 inhibits all of the MMPs but preferentially binds progelatinase B. TIMP-2 inhibits gelatinase B, however it preferentially binds to progelatinase A and inhibits other members of the MMP family (Howard et al. 1991 -a, Howard et al. 1991 -b). The most recently discovered TIMP, TIMP-3 has been show to be expressed in a broad range of tissues as was found with TIMPs 1 and 2 (Apte et al. 1994, Leco et al. 1994, Silbiger et al. 1994). The biochemistry of TIMP-3 interactions with the MMP family members has yet to be addressed. Currently TIMP-2 is being tested for clinical efficacy in treating human cancers that exhibit high rates of metastatic spread (Parkins 1994). Thus TIMPs represent a growing family of broad spectrum MMP inhibitors that play a critical role in the regulation of ECM degradation/remodeling.

MMPs AND CANCER

MMP Expression in Neoplasia

The MMPs have long been associated with malignant transformation. Stromelysin-1 and gelatinase A were originally cloned from viral or oncogene transformed cells (Collier et al. 1988 , Matrisian et al. 1985). Many oncogenes have been shown to regulate MMP levels, and to date all but one MMP family member has been shown to have an AP-1 transcription element in their promoter (Gaire et al. 1994) and references within) which has been shown in several cases to be involved in mediating this response (Gutmann and Wasylyk 1990 for review). MMPs can also be induced by many growth factors and cytokines in cultured cells, including EGF, PDGF, TNFα and TGFα (for review see Matrisian and Hogan 1990). Although MMPs are induced by oncogenes in several cell types, in situ hybridization studies have demonstrated that most MMPs are produced by normal stromal cells surrounding the tumor (Basset et al. 1990 , Newell et al. 1994 for example), suggesting that tumor produced growth factors may be involved in MMP induction in vivo. Matrilysin is unusual among the MMPs in that it is primarily expressed by epithelial cells and tumor cells of epithelial origin (McDonnell et al. 1991, Pajouh et al. 1991 , Rodgers et al. 1993). In early neoplastic events this segregation of MMP production appears to remain intact, i.e. glandular epithelial derived carcinoma cells express matrilysin while several other MMPs are expressed in the surrounding connective tissue cells (Newell et al. 1994 for example). However, in some late stage squamous cell carcinomas stromelysin-1 and collagenase can be expressed by epithelial derived tumor cells (Wright et al. 1994 and unpublished data), potentially reflecting the epithelial/stromal conversion that occurs frequently in late-stage carcinogenesis.

Tumor Invasion and Metastasis

The process by which neoplasms metastasize is composed of a complex series of events (Fidler 1991 , Khokha et al. 1989 , Liotta et al. 1991 , Nicolson 1989). Liotta has proposed a three step model of tumor cell invasion; tumor cell attachment to the

BL, localized proteolysis of the BL, and migration through the BL and stroma (Liotta 1986). In the case of glandular epithelia, many tissues contain a basal cell layer next to the BL. As carcinoma in situ develops, these basal cells are displaced and the carcinoma cells come in contact with and attach to the BL. The degradation of the BL through the secretion of proteinases can be mediated by not only metalloproteinases and serine proteinases as described previously, but lysosomal cathepsins of both the cysteine and aspartyl class can also be secreted by tumor cells and degrade components of the BL (Kane and Gottesman 1990, Rochefort et al. 1990). Finally the tumor cells migrate through the digested BL and into the ECM with subsequent access to the microvasculature and lymph systems. This three step process of tumor cell invasion is one critical portion of the larger process of metastasis which includes cell motility, intravasation, immune system evasion, extravasation and tumor colony formation (Liotta et al. 1991 , Nicolson 1991).

The evidence for MMP involvement in invasion and metastasis comes from three main sources: correlative evidence demonstrating the expression of MMPs in advanced-stage tumors (discussed in detail below), in vitro models of invasion, and in vivo models of invasion and metastasis. The best evidence of the role MMPs perform in invasion and metastasis have come from studies using the TIMPs as a method of inhibiting all MMP family members. Using in vitro models of invasion with modified Boyden chambers and a membranous barrier of human amnion, reconstituted BL (matrigel), or smooth muscle cells, TIMP-1 (DeClerck et al. 1991) has been shown to inhibit tumor cells from entering or crossing the membrane. When TIMP-1 levels in a nontumorigenic fibroblast cell line were reduced by antisense RNA, the resulting cells were tumorigenic and were able to form distant metastases when injected subcutaneously into mice (Khokha et al. 1989). Mice that were treated i.p. with TIMP-1 every 12 hours and injected i.v. with B16-F10 melanoma cells had fewer experimental metastases (Schultz et al. 1988). TIMP-2 over-expression in metastatic H-ras transformed rat embryo cells inhibited their ability to invade the surrounding tissue and

partially inhibited experimental metastasis (DeClerck et al. 1992).

Although the effect of MMPs and TIMPs on tumor metastasis has been presumed to be related to the ability of the cell to degrade microvascular basement membranes and extravasate, recent evidence points to a role in tumor growth. Using intravital microscopy, B16-F10 cells transfected with TIMP-1 have been shown to extravasate as efficiently as controls, however they have a decreased growth potential at the new site and form fewer metastatic tumors in a chick embryo assay (Khokha et al. 1992, Koop et al. 1994). Another highly metastatic melanoma cell line, M24met, that expresses collagenase and gelatinases A and B has been transfected with TIMP-2 (Montgomery et al. 1994). These cells have reduced growth potential in vivo and in in vitro collagen gels, but their metastatic ability remains the same. Transfection based expression of matrilysin in SW480 colon cancer cells increased the cells tumorgenicity when implanted into the cecum of nude mice, where as in SW620 cells, antisense ablation of endogenous matrilysin mRNA expression decreases tumorgenicity compared to parental cell line controls (Witty et al. 1994). The mechanism underlying the growth effects of MMPs is unclear; it may be an indirect effect through alterations in cellular morphology, or degradation of ECM and BL proteins by MMPs may release growth factors that have been sequestered in the matrix. A number of groups have shown that fragments of matrix proteins can alter gene transcription via interaction with the integrin family of cell surface receptors (Damsky and Werb 1992, Tremble et al. 1992 , Yamamoto et al. 1994) and similar signal transduction mechanisms could stimulate proliferation. Thus MMP expression may play a number of roles in the production, proliferation and metastatic spread of neoplastic cells.

The role of MMPs in metastasis has been more difficult to verify when the system is reduced to altering the expression of a single MMP. The redundancy in number and overlapping substrate specificity of the MMPs has been problematic in the determination of the function of a specific MMP during the metastatic process. Transgenic mice with stromelysin-1 under the control of either the MMTV promoter or

the whey acidic protein promoter show an altered branching phenotype during mouse mammary gland development (Sympson et al. 1994, Witty et al. 1994), but mammary tumors induced in these mice do not appear to be significantly more aggressive (Witty et al. 1994). However, the expression of matrilysin in tumor cells lacking this MMP has been shown to effect BL penetration (Powell et al. 1993) and tumorgenicity (Witty et al. 1994). Although studies with TIMP suggest that the MMP family functions in invasion and metastasis, its individual members alone may not be sufficient to alter the entire metastatic phenotype in vivo. In tumors that express an MMP there tend to be several MMPs that are up-regulated (Basset et al. 1990 , Newell et al. 1994 , Pajouh et al. 1991 for example), so it may be the interaction of several MMPs that function in increasing the metastatic ability of tumor cells.

Other Effects of MMPs Influencing Tumorigenesis

Metalloproteinases also contribute to tumor progression by their involvement in the process of angiogenesis. Most studies focusing on angiogenesis have used TIMPs and/or synthetic MMP inhibitors to block angiogenesis in vitro and in vivo. An in vitro angiogenesis model using umbilical vein endothelial cells and type I collagen gels has shown that TIMP-1 and BB-94 (a synthetic MMP inhibitor) can inhibit the phorbol ester induced formation of vascular like structures in the these gels (Fisher et al. 1994). A recent study investigated the ability of TIMP-2 to inhibit Kaposi's sarcoma (KS) induced neovascularization (Albini et al. 1994). The study showed that TIMP-2 could inhibit vascularization of injected Matrigel combined with either conditioned media from KS cells or βFGF. The interaction of matrilysin and prourokinase is of potential interest in relationship to angiogenesis. Matrilysin can cleave prourokinase such that the amino terminal fragment (ATF) which binds the urokinase cell surface receptor is released from high molecular weight prourokinase (Marcotte et al. 1992). The ATF has had a number of activities linked to it, including increasing cell motility and mitogenic stimulation. The ability to increase chemotactic cell motility has been shown for

epithelial cells (Del Rosso et al. 1993), endothelial cells (Odekon et al. 1992) and fibroblasts (Anichini et al. 1994). Thus the production of ATF by tumor cells could effect tumor cell migration as well as neovascularization of the tumor mass.

MMPs have been linked to the process of apoptosis (programmed cell death) which is an important developmental process that can be exploited during the treatment of hormone dependent cancer. Prostate and mammary glands undergo apoptotic cell death during castration induced involution and post-lactation involution respectively (Tenniswood et al. 1992 for review). Matrilysin is expressed in the epithelial cells of prostatic ducts during the involution of the rat ventral prostate (Powell et al. 1995). In situ hybridization indicates that matrilysin is not produced by the secretory epithelial cells undergoing apoptosis, but rather is produced in the cuboidal epithelium that lines the 1° and 2° ducts leading to the urethra (unpublished data). There are preliminary indications that androgen responsive prostate cell line LNCaP produces matrilysin in response to TPA induced apoptosis (Bowden, personal communication). In the involuting mammary gland, stromelysin-3 is expressed by the fibroblasts surrounding the degenerating ducts (Lefebvre et al. 1992). Two independent transgenic mouse models of stromelysin over-expression in mammary epithelial cells indicate that degradation of the BL leads to an increase in epithelial cell apoptosis (Boudreau et al. 1995, Witty et al. 1995). These studies suggest the possibility that during apoptosis, the MMPs degrade the BL and disrupt the interaction between the epithelial cells and their normal substratum, potentially leading to the degeneration of ductal structures and wide spread tissue remodelling.

Scope of MMP Expression and Cancer

MMP expression has been examined in numerous tumors by three primary methods; northern, in situ hybridization and zymographic analysis. Both northern analysis and in situ measure mRNA content, however northern analysis is more quantitative and in situ hybridization is used to localize the mRNA to a particular cell

type. Zymography uses a substrate impregnated SDS-PAGE gel to assess protein level. Table 2 is a list of the MMPs and the neoplasms that have been shown to express the indicated MMP by one of the previously described methods (Table 2). Breast, colon and prostate cancers are among the most completely described systems and will be discussed in more detail below.

Breast cancer has been receiving more attention as of late due to a rising incidence and increased public awareness (Boring et al. 1993). Metastatic spread to the axillary lymph nodes is an important prognostic tool as the number of lymph nodes that are positive for metastatic colonies is directly related to the patient's survival (Harris et al. 1993). A number of MMPs have been associated with metastatic breast cancer, however only stromelysin-3 has thus far been associated with grade/stage of the tumor. This MMP was originally cloned from a mammary carcinoma and found to be expressed by the stromal cells surrounding the tumor (Basset et al. 1993). To date, stromelysin-3 has been found in virtually all metastatic beast cancers studied (95 to 97%) (Basset et al. 1994, Kawami et al. 1993). When stromelysin-3 expression by in situ hybridization was compared to patient survival a significant positive correlation was found with fatal metastatic disease (Engel et al. 1994). The question arises, if ECM proteins are poor substrates for stromelysin-3 then what effect does stromelysin-3 have on tumor cell invasion and metastasis? The answer may not be in the ECM substrates but in other proteins that function in the metastatic process. Stromelysin-3 has been shown to proteolytically inactivate alpha 1-proteinase inhibitor, a serine proteinase inhibitor (Pei et al. 1994). This activity fits with the previously discussed role for both stromelysin-3 and MT-MMP in not degrading the matrix, but to aid the invasive phenotype by activating other proteinases or inactivating inhibitors of proteinases involved in invasion. Further evidence that activation of MMPs is an important step in breast cancer are a number of in vitro and in vivo studies showing activation of gelatinase A in breast cancer (Azzam et al. 1993, Brown et al. 1993, Noël et al. 1994, Thompson et al. 1994). It is known that normal mammary epithelial cells are highly

dependent on interactions with the BL and myoepithelial cells for maintaining normal mammary functions and morphology (Sympson et al. 1993, Talhouk et al. 1992). Consequently, neoplastic transformation of mammary epithelial cells and expression of MMPs by both the tumor and stromal cells may aid in the further phenotypic changes associated with mammary cancer.

Colon cancer has been one of the most studied neoplasms, and as a consequence a very defined set of genetic alterations have been shown during the progression from normal mucosa to malignant colon cancer (Fearon and Vogelstein 1990). McDonnell et.al examined the expression of MMPs by northern analysis and found significant levels of matrilysin mRNA (McDonnell et al. 1991). A thorough study of MMP expression by in situ hybridization in colon cancer was presented by Newell et.al (Newell et al. 1994).This study indicated that matrilysin was expressed exclusively in tumor epithelium and was expressed focally in 8 of 17 early adenomas (<1.0 cm in diameter) and at higher levels in 9 of 10 late-stage carcinomas. Stromelysins 1 and 3 and gelatinase A are all seen exclusively in the stroma of carcinomas, but not all MMPs are expressed within the same tumor (Newell et al. 1994). Witty et.al have provided evidence that matrilysin may have an effect on colon cancer cell growth in an orthotopic model of colon cancer. SW480 human colon cancer cells were transfected with the matrilysin cDNA under a constitutive promoter and when these cells were implanted into the cecum of nude mice the tumors that arose were faster growing than nontransfected SW480 cells(Witty et al. 1994). In the matched cell line SW620 (SW480 from the primary tumor and SW620 from a lymph node metastasis) which constitutively secretes matrilysin, antisense ablation of the matrilysin mRNA reduced the number of tumors (Witty et al. 1994). Thus the expression patterns during colon tumor progression suggest a role for matrilysin in early stages of colon cancer, i.e. tumor cell growth, whereas the MMPs expressed by stromal cells may play a role in later stage events such as tumor cell invasion and metastasis.

Prostate cancer is a slow growing neoplasm believed to arise from the epithelial cells of the gland. Given its deep anatomical location and slow growth rate, its symptomatic onset often allows for metastatic spread prior to diagnosis. Matrilysin and gelatinase A were found to be over expressed in human prostate adenocarcinomas compared to normal prostates from young accident victims (Pajouh et al. 1991). This study found that matrilysin was localized to the epithelial component of invasive adenocarcinomas in 72% of cases and 27% of normal controls. Gelatinase A expression by northern analysis was seen in 60% of adenocarcinomas and not detected in normal prostate tissue. Gelatinase B has also been shown to be expressed in invasive malignant prostate cancer by both zymography and western blotting (Hamdy et al. 1994). In an attempt to understand the functional role for MMP expression in prostate adenocarcinomas, the matrilysin cDNA was transfected into the nonmetastatic weakly invasive prostate tumor cell line DU-145 (Powell et al. 1993). When these cells and control transfected cells were injected i.p. into SCID mice, tumor colonies formed on the diaphragm. The diaphragm was removed and cross sectioned and the extent of invasion examined. The cells transfected with the matrilysin gene invaded past the BL in 66.7% of the diaphragms compared to 11.1% for the control transfected cells. In this model no increase in metastatic ability was seen. This data indicated that matrilysin plays a role in early events in invasion, primarily in degrading the BL as discussed previously. Recent data from our laboratory shows that matrilysin is expressed in the post-castration involuting rat ventral prostate (Powell et al. 1995). This is important to a discussion of prostate cancer because chemical or physical castration is one of the primary modes of treatment for metastatic disease (Hanks et al. 1993). The potential implication is that castration of the patient may cause a proteolytic burst of not only matrilysin but also urokinase over a period of days from the tumor cells (Andreasen et al. 1990). This consequence would further spread the already metastatic prostate cancer cells and potentially hasten the patient's disease.

Breast, colon and prostate cancer are among the most common cancers

diagnosed in the Western world and as such have been extensively studied with regards to clinical progression. These three neoplasms whose progression to the malignant phenotype may be linked to the expression of MMPs are just a sample of the wide range of neoplasias that have been investigated. As indicated in Table 2, many other neoplasms have been shown to express MMPs during some point during tumor development.

CONCLUSIONS

The matrix metalloproteinase family continues to expand with the cloning of two new MMPs in 1994 and the recent cloning of TIMP-3. These new additions add more complexity to the field, while at the same time opening new avenues of research. The cloning of MT-MMP and analysis of its cell surface activity will undoubtedly lead to further investigations into cell membrane proteolysis mediated by MMPs. The identification of the furin recognition site in stromelysin-3 and MT-MMP and the activation of stromelysin-3 by furin has indicated that intracellular trafficking and processing of MMPs is an important area of research that needs more attention. The solution of the MMP active site structure will aid in the study of MMP biochemistry and allow for the design of specific inhibitors of each MMP. The increasing complexity of the structure and functions of the MMPs prompted the new MMP classification system. It is becoming apparent that one must think of each MMP member not only in terms of its substrate specificity, but also what other effects it may have on the phenotype of a cell. The classification of the MMPs by protein structure rather than substrate specificity may free the MMPs from the view that they are enzymes that only degrade ECM and BL.

The expression of MMPs by both tumor and stromal cells can be linked to a number of inducing agents including growth factors, oncogenes and integrin signaling. The initiation of constitutive MMP expression by tumor and stromal cells may function to destabilize the ECM and BL surrounding the invading tumor cells, thus allowing

access to the circulatory and lymphatic systems and subsequent distant metastasis. MMPs appear to have other effects on tumorigenesis such as increased angiogenesis and proliferative potential of the tumor cells. Thus the metalloproteinases are not limited to simply degrading structural proteins that surround the cell, but appear to have a more generalized role in the interactions of cells with their matrix environment, affecting basic cellular processes such as differentiation, proliferation and apoptosis. Such important processes regulated by a multigene family of enzymes with overlapping activities implies that the MMPs play a critical role in maintaining normal cellular homeostasis and enhances the observations that misregulation of these proteinases can have important consequences in the neoplastic process.

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Table 1. Classifications of matrix metalloproteinases.

ENZYME	DOMAIN STRUCTURE	SUBSTRATES	ACTIVATION OF OTHER MMPs
Minimal Domain MMP			
Matrilysin[1] (Pump-1, MMP-7)		Proteoglycans[2], Laminin[3], Fibronectin[4], Gelatins[4], Collagen IV[3],Elastin[5], Entactin[6], Tenascin[7]	Interstitial Collagenase[4], Gelatinase B[8]
Hemopexin Domain MMPs			
Interstitial Collagenase[9] (MMP-1)		Collagens I[10], II[10], III[10], VII[11], X[12], Gelatins[10]	Unknown
Neutrophil Collagenase[13] (MMP-8)		Collagens I[14], II[14], III[14]	Unknown
Collagenase-3[15] (MMP-13)		Collagen I[15]	Unknown
Metalloelastase[16] (MMP-12)		Elastin[16]	Unknown
Stromelysin-1[17] (Transin, MMP-3)		Proteoglycans[18], Laminin[18],Fibronectin[18], Collagen III[19], IV[18], V[19], IX[20], Gelatins[18]	Interstitial Collagenase[19], Neutrophil Collagenase[21], Gelatinase B[22]
Stromelysin-2[1] (Transin-2, MMP-10)		Proteoglycans[23], Fibronectin[19], Collagen III[19], IV[19], V[19], Gelatins[19]	Neutrophil Collagenase[24]
Stromelysin-3[25] (MMP-11)		Laminin[26], Fibronectin[26] (Very weakly)	Unknown
Transmembrane Domain MMP			
MT-MMP[27] (MMP-14)		Unknown	Gelatinase A[27]
Fibronectin Domain MMPs			
Gelatinase A[28] (72kD gelatinase, MMP-2)		Gelatins[28], Collagens IV[28], V[28], VII[28], X[29], Elastin[30], Fibronectin[28]	Unknown
Gelatinase B[31] (92kD gelatinase, MMP-9)		Gelatins[32], Collagens IV[31], V[31], Elastin[30]	Unknown

FN Fibronectin like domain | F Furin recognition sequence | H Hinge domain | TMD Transmembrane domain | Col Collagen domain

[1]Muller et al. 1988, [2]Murphy et al. 1991, [3]Miyazak et al. 1990, [4]Quantin et al. 1989, [5]Murphy et al. 1991, [6]Sires et al. 1993, [7]Siri et al. 1995, [8]Crabbe et al. 1992, [9]Goldberg et al. 1986, [10]Miller et al. 1976, [11]Seltzer et al. 1989, [12]Schmid et al. 1986, [13]Hasty et al. 1990, [14]Hasty et al. 1987, [15]Freije et al. 1994, [16]Shapiro et al. 1992, [17]Matrisian et al. 1986, [18]Chin et al. 1985, [19]Nicholson et al. 1989, [20]Okada et al. 1989, [21]Knäuper et al. 1993, [22]Ogata et al. 1992, [23]Fosang et al. 1991, [24]Knauper et al. 1994, [25]Basset et al. 1990, [26]Murphy et al.1993, [27]Sato et al. 1994, [28]Collier et al. 1988, [29]Welgus et al. 1990, [30]Senior et al. 1991, [31]Wilhelm et al. 1989, [32]Moll et al. 1990.

Table 2. Metalloproteinase expression in human tumors.

Metalloproteinase	Neoplasia	Localization	References
Matrilysin	Prostate	Tumor	Pajouh et al. 1991 , Powell et al. 1993
	Colon	Tumor	McDonnell et al. 1991, Newell et al. 1994 , Witty et al. 1994 , Yamamoto et al. 1994
	Head & Neck	ND	Muller et al. 1991
	Breast	Tumor	Basset et al. 1990
	Gastric	Tumor	McDonnell et al. 1991
	Basal Cell Carcinoma	Tumor	Karelina et al. 1994
Interstitial Collagenase	Gastro-intestinal	Stroma	McDonnell et al. 1991, Okada et al. 1995
	Head & Neck	Stroma/Tumor	Okada et al. 1995, Polette et al. 1991
	Breast	Stroma	Okada et al. 1995
Collagenase-3	Breast	Tumor	Freije et al. 1994
Stromelysin -1	Colon	Stroma	Newell et al. 1994
	Squamous cell carcinoma SSC	Stroma/Tumor	Wright et al. 1994
	SSC Esophagus	Tumor	Shima et al. 1992
	Basal Cell Carcinoma	Stroma	Majmudar et al. 1994a
	Head & Neck	ND	Muller et al. 1991
	Brain	ND	Nakano et al. 1993
Stromelysin-2	Head & Neck	Stroma/Tumor	Muller et al. 1993, Polette et al. 1991
Stromelysin-3	Breast	Stroma	Basset et al. 1993 , Engel et al. 1994, Hähnel et al. 1993
	Basal Cell Carcinoma	Stroma	Majmudar et al. 1994b, Wagner et al. 1992 , Wolf et al. 1992
	Head & Neck	Stroma	Muller et al. 1993, Okada et al. 1995
	Colon	Stroma	Newell et al. 1994 , Okada et al. 1995
	Lung	ND	Urbanski et al. 1992
MT-MMP	Breast	Stroma	Okada et al. 1995
	Colon	Stroma	Okada et al. 1995
	Head & Neck	Stroma	Okada et al. 1995
Gelatinase A	Cervix	Stroma/Tumor	Nuovo et al. 1995
	Colon	Stroma	Newell et al. 1994
	SSC Esophagus	Tumor	Shima et al. 1992
	Lung	Stroma/Tumor	Brown et al. 1993 , Nakagawa and Yagihashi 1994
	Prostate	ND	Pajouh et al. 1991
	Thyroid	Tumor	Campo et al. 1992
	Breast	Stroma	Okada et al. 1995
Gelatinase B	Cervix	Stroma/Tumor	Nuovo et al. 1995
	Lymphoma	Tumor/Macrophage	Kossakowska et al. 1993
	Prostate	ND	Hamdy et al. 1994
	Brain	ND	Rao et al. 1993
	Lung	Stroma/Tumor	Urbanski et al. 1992

ND= Not Determined